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REMARKS

STATUS OF THE CLAIMS

Claims 7-20 and Claims 34-38 remain in the application.

The Office rejected claims 7, 20, 37, and 38 under 35 U.S.C. 102(b) as being anticipated by *Behar*.

The Office rejected claims 7-20 and 34-38 under 35 U.S.C. 102(a) as being anticipated by *Morgan*.

SUMMARY OF THE INVENTION

The present invention is directed a method for vaporizing a liquid or solid sample using a micropyrolyzer comprising a substrate having a suspended membrane formed thereon and a resistive heating element disposed on the membrane for heating the sample. The micropyrolyzer can be constructed from semiconductor materials. The sample size can be less than 3 microliters and the heating rate can be 20 - 70°C per millisecond with very low power consumption.

SUMMARY OF THE ART

Behar, U.S. 4,710,354, discloses a method and device for heating of solid or liquid samples in small quantities comprising a sample holding rod that can be inserted into a tubular heated liner for pyrolysis of the sample.

Morgan et al., discloses Applicants' invention.

ARGUMENTS

CLAIMS 1-6 AND 21-33

In Applicants response, dated 1/30/04, to the prior Office Action, Applicants withdrew claims 1-6 and 21-33 from prosecution in response to a restriction requirement. Applicants request that claims 1-6 and 21-33 be canceled.

CLAIMS 7, 20, 37, AND 38, LIMITED TO A MICROPYROLYZED COMPRISING A SUBSTRATE SELECTED FROM THE GROUP CONSISTING OF SEMICONDUCTORS AND DIELECTRICS, ARE NOT ANTICIPATED BY *Behar* UNDER 35 U.S.C. § 102(b)

The Office rejected claims 7, 20, 37, and 38, asserting that the Applicants' method for vaporizing a liquid or solid sample is anticipated by *Behar's* method and device for heating of solid or liquid samples for pyrolyzing a sample. To anticipate a claim, the reference must teach each and every element of the claim. *See* MPEP 2131.

Applicants request that claim 34 be canceled and that claim 7 be rewritten to include the limitation of claim 34, directed to a substrate selected from the group consisting of semiconductors and dielectrics. Applicants request that claim 35 be amended to depend from claim 7. Applicants argue, *infra*, that claim 34 is allowable and that the requested amendment of claims 7 and 35 will place these claims in condition for allowance, or in better form for consideration on appeal. Applicants submit that claims 20, 37, and 38 that depend from and further define claim 7, are also in condition for allowance. *See* MPEP 2143.03.

CLAIMS 7-20 AND 35-38 ARE NOT ANTICIPATED BY *MORGAN et al.* UNDER 35 U.S.C. § 102(a)

The Office rejected claims 7-20 and 34-38, asserting that Applicants' invention was described in *Morgan et al.* within one year before the invention thereof by Applicants. Applicants can rebut the 35 U.S.C. 102(a) *prima facie* case by showing the reference's disclosure was derived from Applicants' own work. The rejection can be overcome by submission of a specific declaration by applicant establishing that the article is describing applicant's own work. *See* MPEP2132.01.

The *Morgan* reference was published in February, 2001 (please note that the Information Disclosure Statement inadvertently listed an incorrect publication date for this reference). The *Morgan* reference was co-authored by co-inventors Mowry, Morgan, Manginell, and Frye-Mason; and by non-inventors Kottenstette and Lewis. Applicants include herewith a declaration by Mowry and Manginell establishing that the *Morgan* reference describes Applicants' own work and that Kottenstette and Lewis are not co-inventors. Furthermore,

Applicants submit herewith a declaration by Kottenstette and Lewis disclaiming their inventorship of the invention claimed in the Application. According, Applicants submit that this rejection is overcome and that claims 7-20, and 35-38 are in condition for allowance.

CONCLUSION

Applicants have responded to each and every requirement and urge that claims are now in condition for allowance. Applicants have responded within two months of the mailing date of the final office action. Therefore, Applicants request an advisory action or expeditious processing to issuance.

Respectfully submitted,

Kevin W. Bieg
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CERTIFICATION UNDER 37 CFR 1.8

I hereby certify that this correspondence and documents referred to herein were deposited with the United States Postal Service as first class mail addressed to: Commissioner for Patents, Alexandria, VA 22313-1450 on the date shown below.

Date: 6/10/04 By: Martha Trujillo

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mowry *et al.*	Group:	1743
Serial No.:	10/035,537	Paper No.:	
Filed:	10/23/01	Examiner:	LaToya I. Cross
For:	Micropyrolyzer for Chemical Analysis of Liquid and Solid Samples		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION REGARDING INVENTORSHIP UNDER 37 CFR 1.132 TO OVERCOME CITED PUBLICATION BY MORGAN ET AL.

PURPOSE OF DECLARATION

This declaration is to establish that a reference cited as Morgan *et al.* in the 35 U.S.C. 102(a) rejection in the Final Office Action mailed April 22, 2004 and attached hereto describes Applicants' own work and is the publication of Applicants' own invention.

The persons making this declaration are the co-inventors of the above referenced patent application and co-authors of the Morgan *et al.* reference.

REVIEW OF CIRCUMSTANCES

Claims 7-20 and 34-38 have been rejected under 35 U.S.C. 102(a) as being anticipated by the following reference cited as Morgan *et al.* with an effective publication date of February, 2001.

> Catherine Morgan, Curtis Mowry, Ronald Manginell, Gregory Frye-Mason, Richard Kottenstette, and Patrick Lewis, "Rapid identification of bacteria with miniaturized pyrolysis/GC analysis," in *Advanced Environmental and Chemical Sensing Technology*, Tuan Vo-Dinh, Stephanus Buettgenbach, Editors, Proceedings of SPIE Vol. 4205, pages 199-206 (2001).

FACTS

The authorship heading of the Morgan *et al.* reference enclosed herewith lists co-authors Catherine Morgan, Curtis Mowry, Ronald Manginell, Gregory Frye-Mason, Richard Kottenstette, and Patrick Lewis as being affiliated with Sandia National Laboratories at the time the work described therein was performed. Catherine Morgan is Catherine H. Morgan; Curtis Mowry is Curtis D. Mowry; Ronald Manginell is Ronald P. Manginell; and Gregory Frye-Mason is Gregory C. Frye-Mason, all of whom are co-inventors of the referenced patent application.

The authorship heading of the Morgan *et al.* reference also lists Richard Kottenstette and Patrick Lewis, neither of which are co-inventors of the referenced patent application.

TIME OF PRESENTATION OF THE DECLARATION

This declaration is submitted subsequent to final rejection.

DECLARATION

1. I hereby declare that I am a co-author of the above reference cited as Morgan *et al.*

2. I hereby further declare that the Morgan *et al.* reference describes my own work and the invention for which the patent application referenced herein has been submitted, and of which I am a co-inventor.

3. I hereby declare that Catherine H. Morgan, Ronald P. Manginell, and Gregory C. Frye-Mason are also co-inventors of the invention in the patent application referenced herein.

4. I hereby declare that Richard Kottenstette and Patrick Lewis, although listed as co-authors of the Morgan *et al.* reference, are not co-inventors of the invention in the patent application referenced herein.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

Full name of co-inventor: Curtis D. Mowry

Inventor's signature: Curtis D. Mowry Date: 6/8/04

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mowry *et al.*	Group:	1743
Serial No.:	10/035,537	Paper No.:	
Filed:	10/23/01	Examiner:	LaToya I. Cross

For: Micropyrolyzer for Chemical Analysis of Liquid and Solid Samples

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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PURPOSE OF DECLARATION

This declaration is to establish that a reference cited as Morgan *et al.* in the 35 U.S.C. 102(a) rejection in the Final Office Action mailed April 22, 2004 and attached hereto describes Applicants' own work and is the publication of Applicants' own invention.

The persons making this declaration are co-authors of the Morgan *et al.* reference.

REVIEW OF CIRCUMSTANCES

Claims 7-20 and 34-38 have been rejected under 35 U.S.C. 102(a) as being anticipated by the following reference cited as Morgan *et al.* with an effective publication date of February, 2001.

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FACTS

The authorship heading of the Morgan *et al.* reference enclosed herewith lists co-authors Catherine Morgan, Curtis Mowry, Ronald Manginell, Gregory Frye-Mason, Richard Kottenstette, and Patrick Lewis as being affiliated with Sandia National Laboratories at the time the work described therein was performed. Catherine Morgan is Catherine H. Morgan; Curtis Mowry is Curtis D. Mowry; Ronald Manginell is Ronald P. Manginell; and Gregory Frye-Mason is Gregory C. Frye-Mason, all of whom are co-inventors of the referenced patent application.

The authorship heading of the Morgan *et al.* reference also lists Richard Kottenstette and Patrick Lewis, neither of which are co-inventors of the referenced patent application.

TIME OF PRESENTATION OF THE DECLARATION

This declaration is submitted subsequent to final rejection.

DECLARATION

1. I hereby declare that I am a co-author of the above reference cited as Morgan *et al.*

2. I hereby further declare that I have reviewed Claims 7-20 and 34-38 in the referenced patent application, did not conceive of the inventions recited in any of those claims, and hereby disclaim inventorship of the referenced patent application.

3. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

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Stephanus Büttgenbach
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Rapid identification of bacteria with miniaturized pyrolysis/GC analysis

Catherine Morgan, Curtis Mowry, Ronald Manginell, Gregory Frye-Mason, Richard Kottenstette, Patrick Lewis

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ABSTRACT

Identification of bacteria and other biological moieties finds a broad range of applications in the environmental, biomedical, agricultural, industrial, and military arenas. Linking these applications are biological markers such as fatty acids, whose mass spectral profiles can be used to characterize biological samples and to distinguish bacteria at the gram-type, genera, and even species level. Common methods of sample analysis require sample preparation that is both lengthy and labor intensive, especially for whole cell bacteria. The background technique relied on here utilizes chemical derivatization of fatty acids to the more volatile fatty acid methyl esters (FAMEs), which can be separated on a gas chromatograph column or input directly into a mass spectrometer. More recent publications demonstrate improved sample preparation time with *in situ* derivatization of whole bacterial samples using pyrolysis at the inlet; although much faster than traditional techniques, these systems still rely on bench-top analytical equipment and individual sample preparation.

Development of a miniaturized pyrolysis/GC instrument by this group is intended to realize the benefits of FAME identification of bacteria and other biological samples while further facilitating sample handling and instrument portability. The technologies being fabricated and tested have the potential of achieving pyrolysis and FAME separation on a very small scale, with rapid detection time (1-10 min from introduction to result), and with a modular sample inlet. Performance results and sensor characterization will be presented for the first phase of instrument development, encompassing the microfabricated pyrolysis and gas chromatograph elements.

1. INTRODUCTION

Fatty acids have long been molecules of environmental, biomedical, agricultural, and industrial importance. Because of their high molecular weight and low volatility, they have always been a challenge for the analytical chemist. A common solution has been the use of derivatization reagents to increase their volatility and the most widely utilized derivatization is to convert the fatty acid to a fatty acid methyl ester (FAME).

One method to create FAMEs, using the derivatizing reagent tetramethylammonium hydroxide (TMAH) followed by pyrolysis or rapid heating of the mixture, has been practiced since 1963 [1]. A summary of the reaction is shown in Figure 1. To effect the reaction, a derivatizing agent and heat are required. In this case, tetramethylammonium-hydroxide, a strong base, is mixed with the sample. The first reaction occurs over a matter of seconds at room temperature and yields a salt of the fatty acid and derivatizing reagent. Rapid heating completes the conversion to the fatty acid methyl ester. This method has been shown to work for triglycerides as well.

With respect to the analysis or identification of bacteria, the ability to differentiate one type of microorganism from another using fatty acid content and distribution is well known [2]. A benchtop commercial method of extracting, methylating, and analyzing fatty acid content has been available for some years [3]. The FAME analysis is performed by gas chromatography and the results compared with computer databases to identify possible matches. The analysis takes 15-20 minutes per run, however, not including the extraction and preparation time. The extraction and preparation time can range from one hour to one day.

In order to decrease the analysis time researchers have used the pyrolysis reaction described above directly on bacterial samples using Curie-point pyrolysis. In Curie-point pyrolysis, the sample, including bacterial sample and methylation reagent, is coated on a metallic wire that is heated using a powerful (up to 1 kW) radio frequency generator. The wire heats until its Curie-point temperature is reached, at which point the wire is no longer magnetic and ceases to heat. Curie-point pyrolysis coupled with methylation of whole bacterial cells and GC analysis was performed as early as 1991 [4]. Typically the pyrolysis reaction is carried out in an inert gas such as helium or nitrogen, however it has also been demonstrated in air. The FAME profiles obtained in air were still sufficient to differentiate the bacteria tested [5].

Portable instrumentation being developed for bacterial detection using FAME signals has concentrated to date on using either infrared or resistive heating pyrolysis followed by mass spectral analysis. Both methods use large amounts of power in the pyrolysis step, and the infrared type is slow and not suitable as a chromatographic introduction. The availability of a rapid

Advanced Environmental and Chemical Sensing Technology, Tuan Vo-Dinh, Stephanus Buettgenbach, Editors, Proceedings of SPIE Vol. 4205 (2001) © 2001 SPIE. · 0277-786X/00/$15.00

and low-power pyrolyzer could reduce the size and power required of existing instrumentation. The same can be said for a portable chromatograph. The goal of this work has been to determine whether microfabricated components could facilitate a pyrolysis/methylation reaction and therefore demonstrate the potential for a hand-held FAME sensor.

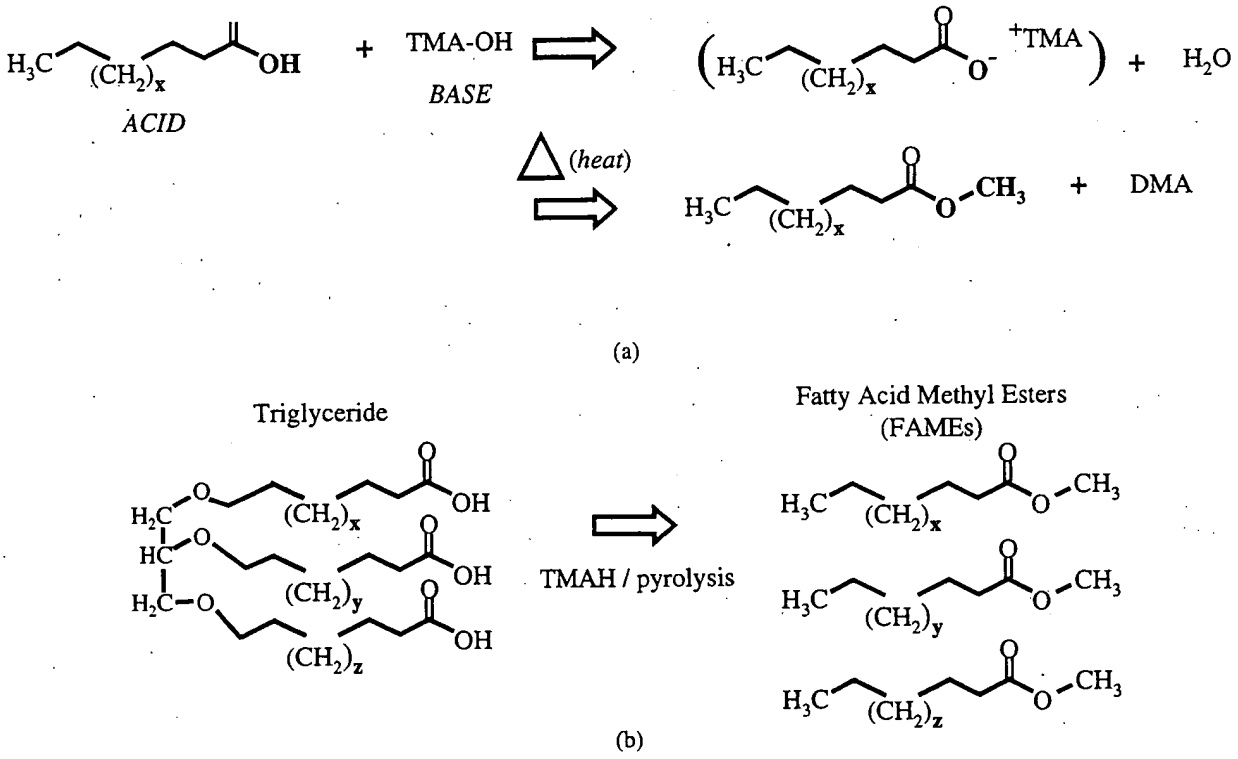

Figure 1. (a) Pyrolysis/methylation reaction of a fatty acid to its representative methyl ester. (b) Pyrolysis/methylation/conversion of a triglyceride to its three representative FAMEs.

A program to develop a portable, autonomous chemical sensor has been ongoing at Sandia National Laboratories. Components of the sensor, described elsewhere as part of the μChemlab® program[6,7], have included a preconcentration stage or mini-hotplate that heats rapidly and has a low heat capacity, a micro-gas chromatograph column, and miniature surface acoustic wave array detectors. In the first phase of this work, the potential for μChemlab components to perform the functions critical to a pyrolytic FAME analysis (pyrolysis and chromatography) was evaluated. The results of that evaluation are the subject of this paper. A block diagram of the envisioned biodetector is shown in Figure 2.

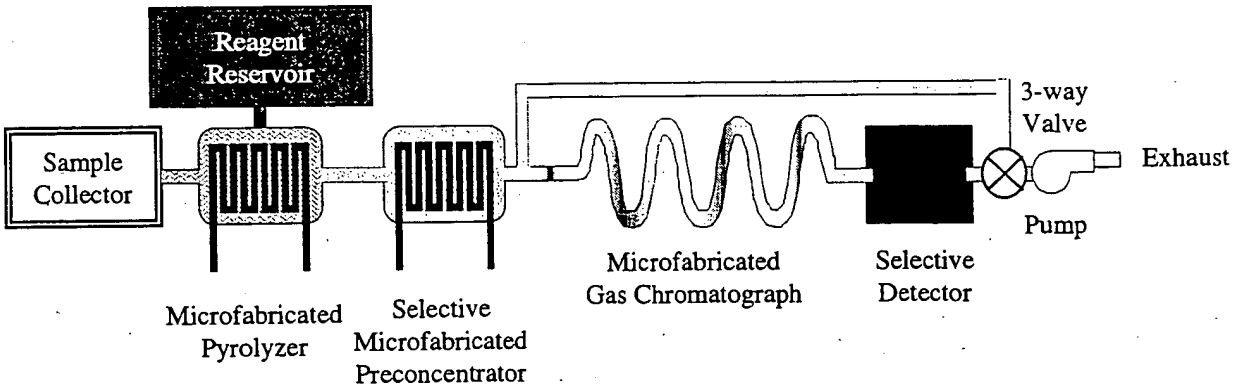

Figure 2. Potential design of a biodetector based upon microfabricated pyrolysis/methylation and gas chromatography.

2. EXPERIMENTAL DETAILS

The design and development of the microfabricated hotplate (micropyrolyzer) and microfabricated gas chromatograph column (microGC) have been described previously [8,9,10,11]. These devices were tested individually here for the application of pyrolysis/methylation of fatty acids and other samples related to detection of bacteria and biological samples of interest.

Purified fatty acids, triglycerides, and fatty acid methyl esters were purchased from Sigma and Supelco and used without further modification. Canola oil was obtained from a standard grocery brand. GC purity TMAH, methanol, and chloroform reagents were purchased from Fisher Scientific; TMAH was used as the methylating agent as described above; methanol and chloroform were used to dilute and solvate the fatty acid and related samples as necessary.

The micropyrolyzer was evaluated using a Hewlett Packard model 5890/5971A MSD gas chromatograph/mass spectrometer (Agilent, Palo Alto, CA). Tests were conducted by injecting samples and reagent directly into the device cavity, followed by a bias pulse to power the resistive heater. A device outlet led to the GC injection port with a heated transfer capillary, swept with helium. A 15 m GC column (J&W Scientific DB23) was used to separate product peaks prior to detection.

The microGC was evaluated using an HP model 6890 gas chromatograph outfitted with a flame ionization detector (FID). Nitrogen was used as the carrier gas. The microfabricated column used was 1 meter long, coated with the stationary phase OV1 (poly(dimethylsiloxane)).

3. RESULTS AND DISCUSSION

3.1 Micropyrolyzer

Tests were conducted separately on the micropyrolyzer and microGC elements. Figure 3 (a) shows photographs of the microfabricated hotplate device. Figure 3 (b) illustrates the device used as a pyrolyzer.

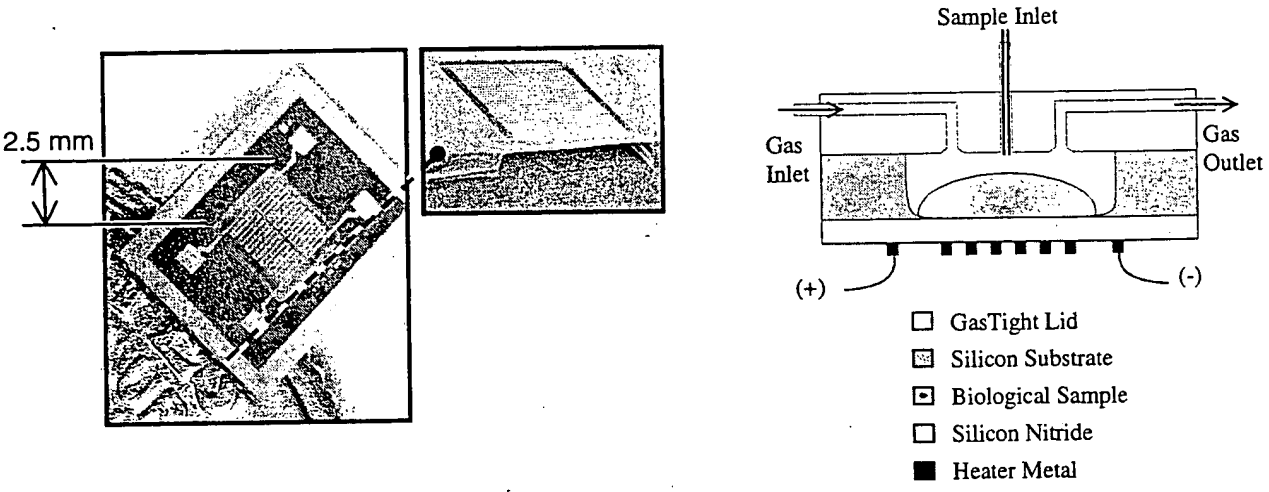

Figure 3. (a) Photographs of the microfabricated hotplate show the metal resistive heater formed on top of the suspended silicon nitride membrane. (b) The device when used as a micropyrolyzer is configured to hold the sample inside the silicon cavity, on the opposite side of the nitride membrane. The test fixture includes a gas tight lid, liquid sample inlet, and carrier gas inlet and outlet.

Initial evaluation of the micropyrolyzer included power pulse tests and IR camera tests to measure the ability to heat the membrane rapidly with a liquid sample load. Devices were able to withstand repeated biasing with repeatable temperature response. Figure 4 shows data collected with the IR camera, demonstrating a temperature ramp from 85 to 290°C in about 60 msec using 250 mW power; temperature ramps up to 70°C/ms have been demonstrated, with larger bias voltage resulting in faster ramps.

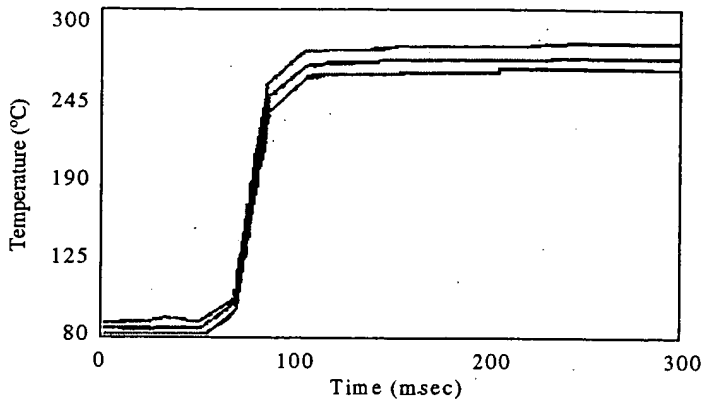

Figure 4. Temperature ramp demonstrating ability of micropyrolyzer to heat rapidly under a sample load. Data was taken with a computer controlled IR camera; device was loaded with 1.5 µl fatty acid solution.

Figures 5 through 9 give results from several tests using the micropyrolyzer to effect pyrolysis/methylation reactions of various samples. FAME peaks are denoted in these figures by the number of carbons and the number of unsaturated bonds (X:Y); for example, the (16:0) peak is the hexadecanoic acid methyl ester (or palmitic acid methyl ester). Fatty acids (FAs) are the simplest precursor for FAME generation so initial tests used purified FAs to evaluate the ability of the device to transform the analyte. Figure 5 shows results from a mix of eight fatty acids in unequal amounts; test results indicate a qualitative transformation of the mix and offer a first time demonstration of pyrolysis using a microfabricated device; this transformation required less than 5 seconds for sample preparation and reaction, after the sample was manually loaded onto the membrane.

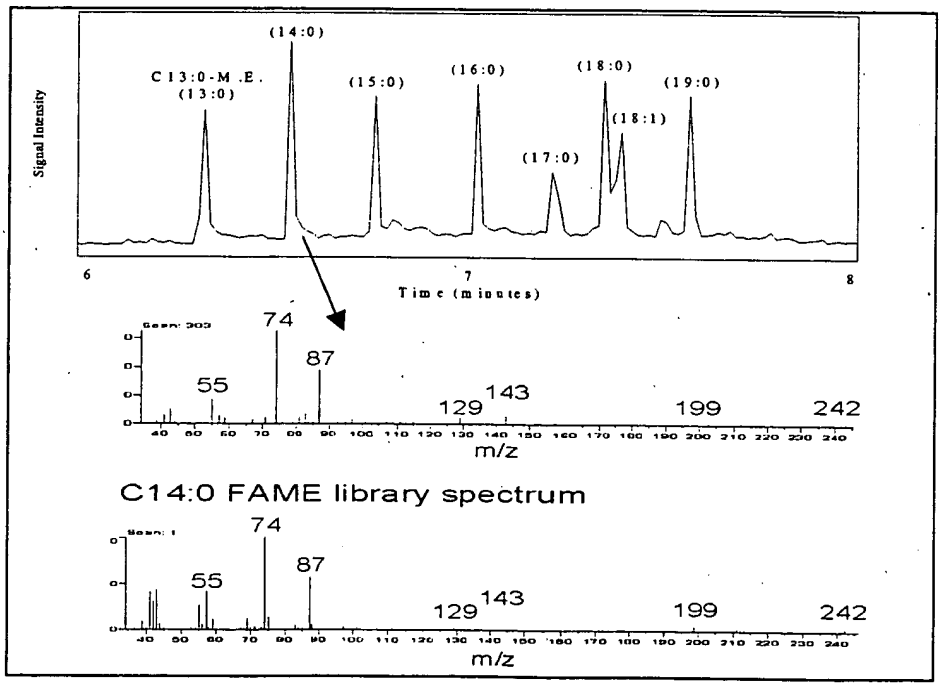

Figure 5. Gas chromatograph/mass spectrometer results from pyrolysis/methylation of a mix of eight fatty acids using the micropyrolyzer. The top graph shows the integrated detector response; the middle plot gives the mass/ion spectrum of the second peak; the third plot shows the spectrum for the C14:0 methyl ester from a mass spectra library database. All peaks had spectra that matched with the expected FAME peak; no additional contamination or FA peaks were found. FAME peaks are denoted in here by the number of carbons and the number of unsaturated bonds (X:Y); for example, the (16:0) peak is the hexadecanoic acid methyl ester.

Figure 6 shows chromatographs from another test where the pyrolysis/methylation reaction of two fatty acid mixes were compared: mix A contained the six FAs C12:C13:C15:C16:C17:C18 (all saturated) with relative concentrations of 4:1:1:4:4:4, respectively; mix B contained the same six FAs with relative concentrations of 1:4:4:1:1:1, respectively; the distribution of percentage concentrations within each mix can be calculated as 22%:6%:6%:22%:22%:22%, for mix A, and 8%:33%:33%:8%:8%:8%, for mix B. These mixes roughly simulate differences in FAME peak signatures seen for *in situ* pyrolysis/MS of two bacteria, *Pseudomonas fluorescens* and *Bacillus cereus*, demonstrated using standard equipment [12].

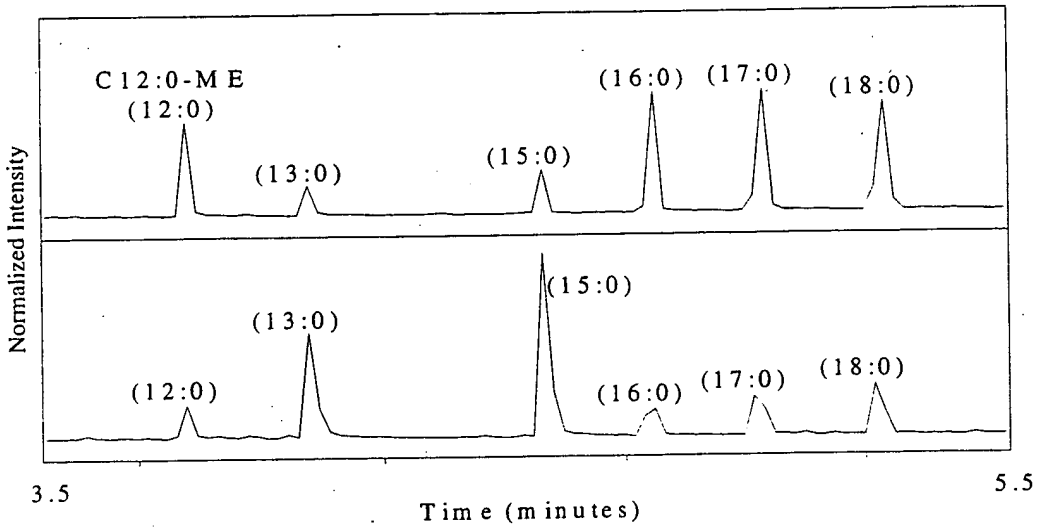

Figure 6. Pyrolysis/methylation of two mixes of fatty acids. Test results show good correlation between initial FA composition and relative areas of the resultant FAME peaks.

Figure 7 shows the same FAME peaks with percentage peak areas calculated, with some deviation form what would be expected if all FAs had converted with the same efficiency. The ability to measure accurate concentrations will depend on consistent conversion yields for each analyte and analyte mix. These preliminary data, coupled with other observations, show that conversion is approximately quantitative but that some loss of product occurs. Improvements in the device design and sample loading should enable us to resolve these conversion issues.

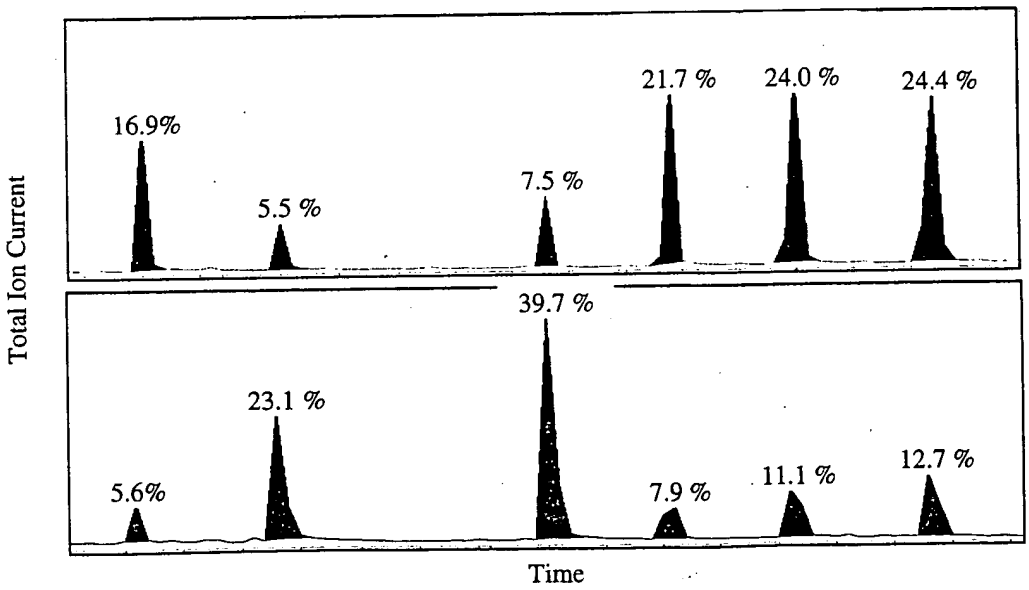

Figure 7. Same data as shown in Figure 6 with peak areas calculated for comparison.

Figures 8 and 9 give results from tests using more complex analytes that contain fatty acids in forms more typical for biological samples. In Figure 8, a mix of five triglycerides was pyrolyzed; this mixture contained equal amounts of tricaprylin, tricaprin, trilaurin, trimyristin, and tripalmitin. As can be seen, five FAME peaks were formed, corresponding to the five FA types found within the triglyceride mix. Conversion efficiency may have been affected by the triglyceride composition of the original analyte; however, effective transformation of a more complex analyte was demonstrated.

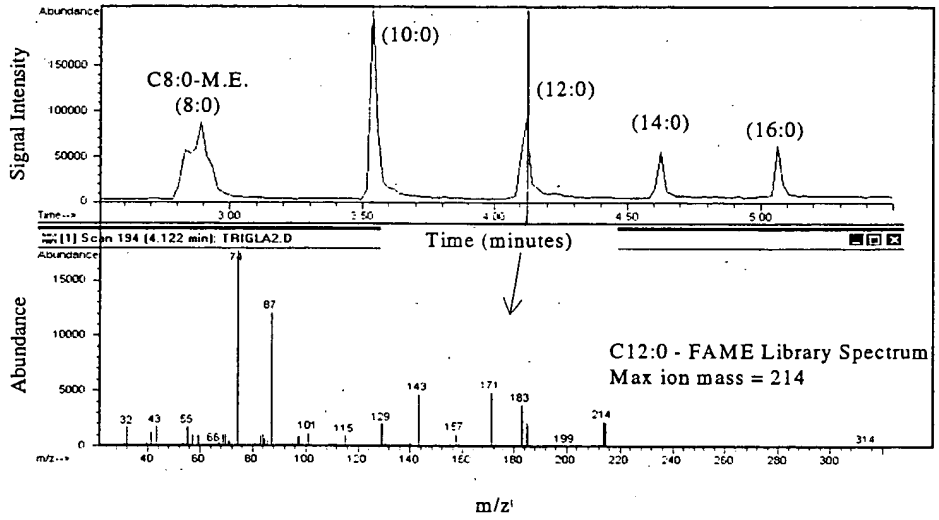

Figure 8. Pyrolysis/methylation of a triglyceride mix, showing effective transformation of the triglycerides into the five types of fatty acid tails.

Figure 9 gives results from a test on another kind of lipid sample, Canola oil; Canola oils are one of many food types with high value fatty acid content including mainly unsaturated fats considered to be more healthy; degradation and alteration of products can change this distribution. This test demonstrates the ability of the reaction achieved with the micropyrolyzer to transform a sample with both saturated and unsaturated components.

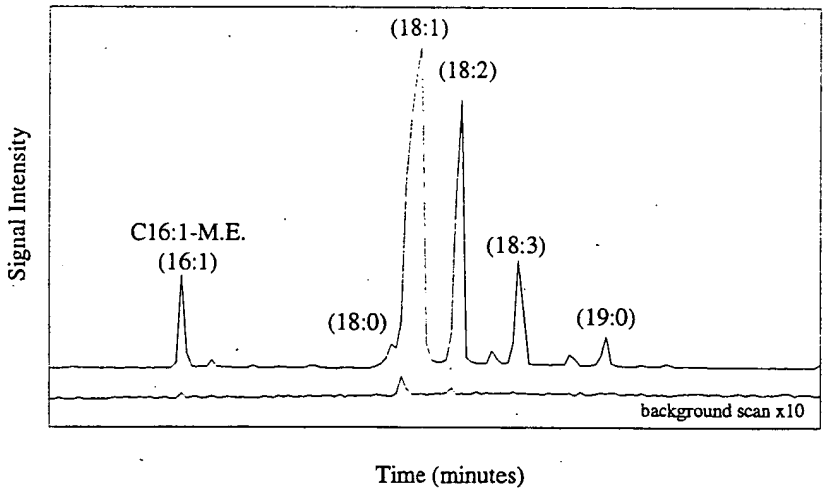

Figure 9. These data show pyrolysis/methylation of a 1 μL sample of Canola oil and demonstrate that the device can efficiently transform and distinguish between closely related peaks of saturated and unsaturated FAMEs. For comparison, a test with oil and heat but no TMAH was conducted; the background scan shows small residual carryover and minute FAME conversion, demonstrating the ability of the TMAH mediated conversion to dominate over competing effects.

3.2 Microfabricated gas chromatograph column

Figure 10 shows optical and SEM photographs of a microfabricated gas chromatograph (GC) column similar to the one used for the test described below.

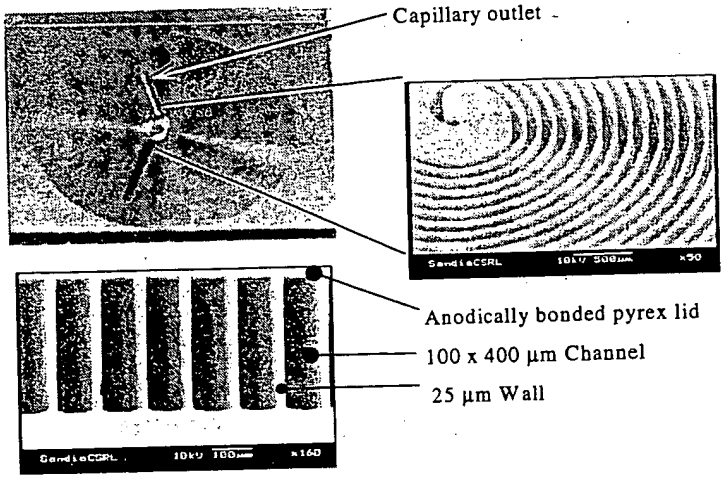

Figure 10. The optical photograph in the top left hand corner shows a single die, cut from a 4" silicon wafer after anodic bonding with a Pyrex plate. Each die is 1.25 x 1.25 cm and contains within it a 1 m long channel. This photograph also shows the outlet capillary tube perpendicular to the surface of the microGC. This spiral is shown in part in the top right hand SEM photograph. The SEM photograph at the bottom of the figure shows a cross-section cut through the device, showing the rectangular cross-section of the channel and the thin walls etched with a deep reactive ion etcher that makes possible Sandia's uniquely small microGC column.

Figure 11 shows results for one of several tests conducted with the microGC columns. For this test, purified FAMEs were injected directly onto the column, which was then temperature ramped from 60 to 150°C. Baseline separation of all 10 FAME peaks was achieved repeatably although a baseline shift with temperature was observed. The column employed here was coated with OV1, a nonpolar stationary phase.

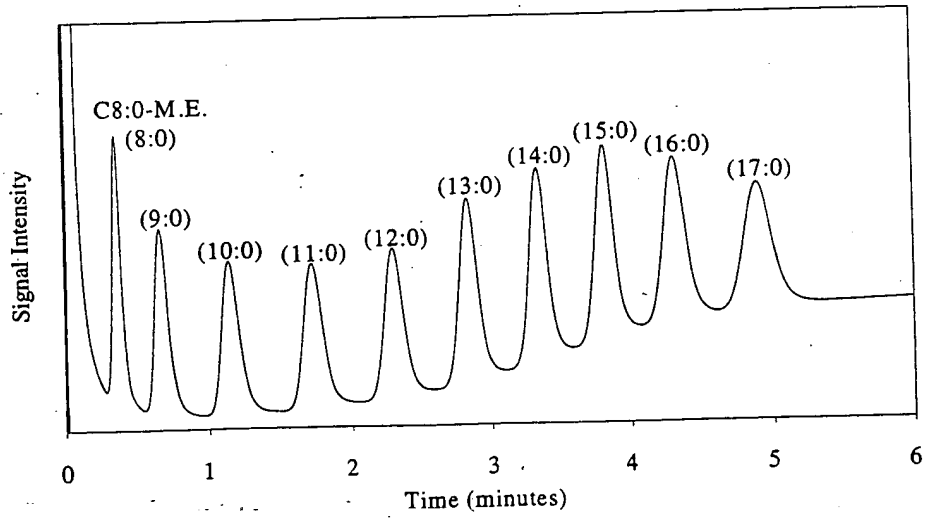

Figure 11. Baseline separation of FAME peaks using the microfabricated GC column.

4. CONCLUSIONS

The performance results of the miniature pyrolyzer and miniature GC demonstrate that the potential exists for a microfabricated sensor to perform a FAME analysis similar to that performed by commercial instrumentation. Such a sensor could find many applications in the environmental, biomedical, agricultural, industrial, and military arenas. The advantages offered by a miniaturized system using microfabricated elements include the possibility of producing a detector that is low power, low cost, hand-held, and lightweight. In addition, the selectivity of these device elements and other components taken from Sandia's μChemLab system is tunable, allowing selectivity against many interferents. This project represents the first published work showing successful pyrolysis/methylation of fatty acids and related analytes using a microfabricated pyrolysis device.

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Keywords: microsensor, GC, gas chromatograph, fatty acid, pyrolysis

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